

[illegible]

FIG. 1C

N288D

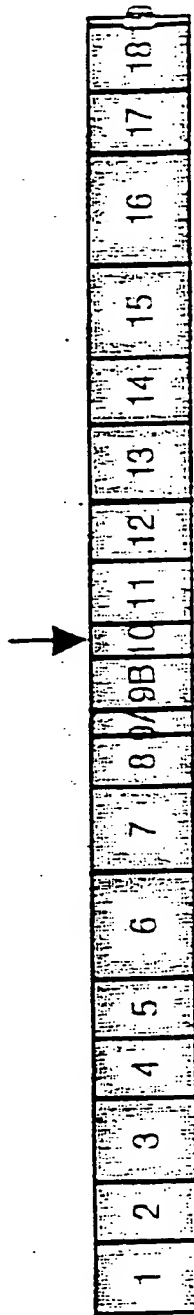


FIG. 3A

GTATGGAACA GGA TGT TTC TTA CTA TGT $\frac{A}{G}$ ATACA GGC CAT AAG

270

310

GK N288D Mutant

glpk_human
glpk_rat
glpk_mouse
glpk_ecoli
glpk_pseae
glpk_entca
glpk_haeln
glpk_bacsu
glpk_yeast
glpk_mycge
glpk_enlfa
glpk_mycpn
glpk_syny3

FQIGQAKNTYGTGCFLLDITGHIKCVFSDHGLITTVAYKLGR SEQ ID NO: 6
FQIGQAKNTYGTGCFLLDITGHIKCVFSDHGLITTVAYKLGR SEQ ID NO: 7
FQDGGQAKNTYGTGCFLLDITGHIKCVFSEHGLITTVAYKLGR SEQ ID NO: 8
FQDGGQAKNTYGTGCFLLDITGHIKCVFSEHGLITTVAYKLGR SEQ ID NO: 9
VKFGMAKNTYGTGCFLLDITGHIKCVFSEHGLITTVAYKLGR SEQ ID NO: 10
VF:PGQAKNTYGTGCFLLDITGHIKCVFSEHGLITTVAYKLGR SEQ ID NO: 11
FEKGMINKNTYGTGAFIVMNTGEEFQISDNDLLTIGY--GI SEQ ID NO: 12
VHAGQAKNTYGTGCFLLDITGHIKCVFSEHGLITTVAYKLGR SEQ ID NO: 13
FEFGMGKNTYGTGCFLLDITGHIKCVFSEHGLITTVAYKLGR SEQ ID NO: 14
YKPGAACKTYGTGCFLLDITGHIKCVFSEHGLITTVAYKLGR SEQ ID NO: 15
TF:PGMVKNNTYGTGCFLLDITGHIKCVFSEHGLITTVAYKLGR SEQ ID NO: 16
FE:PGMVKNNTYGTGCFLLDITGHIKCVFSEHGLITTVAYKLGR SEQ ID NO: 17
VE:PAVMKNTYGTGCFLLDITGHIKCVFSEHGLITTVAYKLGR SEQ ID NO: 18
DRPGLLKCTYGTGAFLVNTGQTVTRSQHRIISTVAVNTQIN SEQ ID NO: 19

FIG. 3B

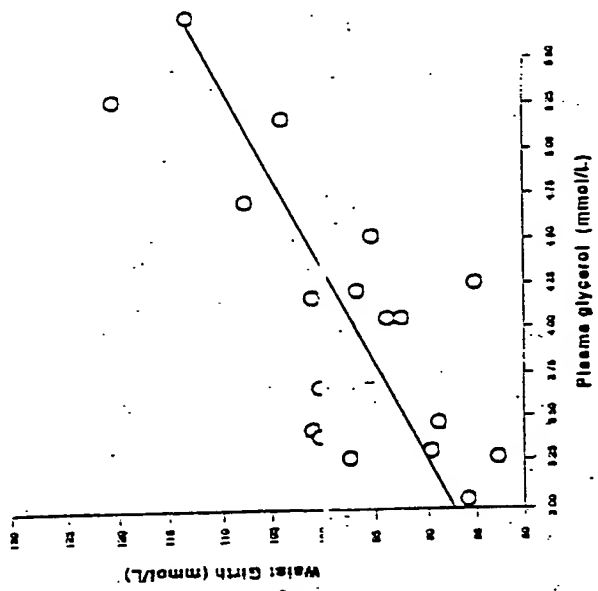


FIG. 4A

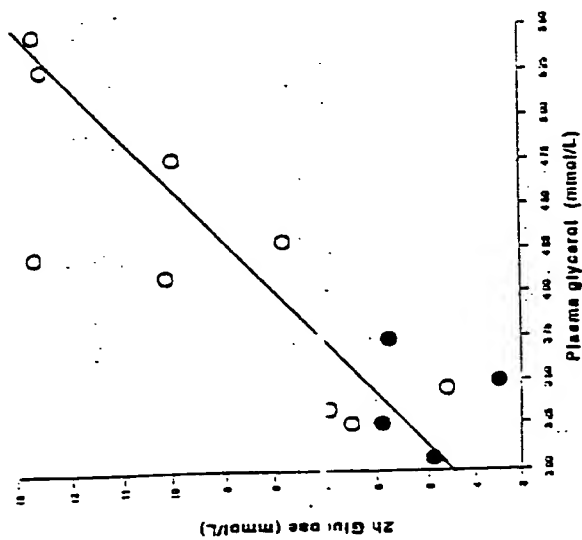


FIG. 4B

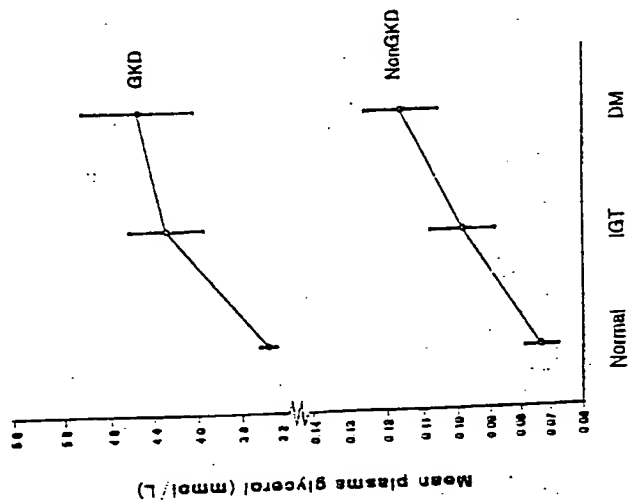
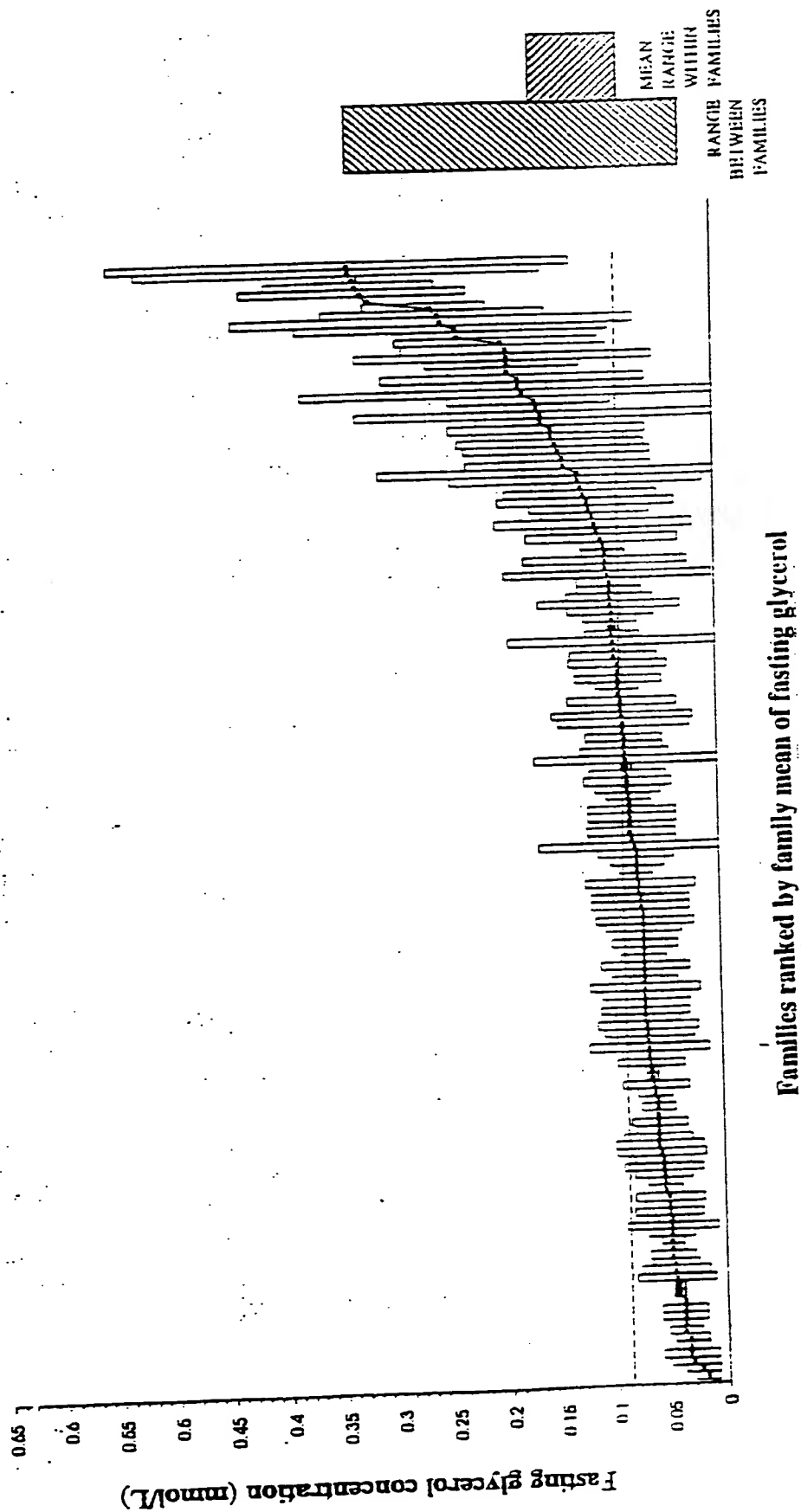


FIG. 4C

FIG. 5



poly: A/G
location: 13th base of exon 3

ATGCCTTCTTTTGTCAAAGATGGGTGGAACA [A/G] GACCCTAAGGAAATTCTACAT
TCTGTCT SEQ ID NO: 1

CAA vs CAG ==> silent

poly: A/C
location: 17th base of intron 8
TAATGGTAAAAACAAACAAA [A/C] AAACAAAAACACACCAAAAAACCAA

SEQ ID NO: 2

poly: A/G
location: 29th base of exon 10

TTCATTCTCCCTTCAACCATAGGTATGGAACAGGATGTTTCTTACTATGT [A/G] AT
ACAGGCCATAAGGTtGGTTTTTAATAAAAATGATTAAGTCA SEQ ID NO: 3

AAT vs GAT ==> N to D

poly: G/T
location: 22nd base of intron 12
GAAATTGGTGAGTGTGTTCTAACAAAAG [G/T] TTAGAAAATCTGAAAAATGACACA
TTTCA SEQ ID NO: 4

FIG. 6

09694088-102000

SEQ ID NO: 5

Exon 1:
GGTTCAGCGGACGCGCGCGGCCCTCGGTCTCTGGA CTGTCACCTGCCCCCTCCCCCTCCCGCC
GCCGTCACCCAGGAAACCGGCCGCAATCGCCGCGCCGACCTGAAGCTGGTTTTCATGGCAGCCT
CAAAGAAGGCAGTTTTGGGGCCATTGGTGGGGGCGGTGGACCAGGGCACCAGTTCGACGCGC
TTTTTGCTGAGCCCGGGGTGACATGTGAAGAGGCGCTGAGC

Exon 2:
TGTA AACGACGGCCAGTCATCCTTGATATCTGCCTGCATTTTTACATTAATATTACAATAT
CTTTTTCAGGTTTTCAATTCAAAAACAGCTGAACTACTTAGTCATCATCAAGTAGAAATAAA
ACAAGAGTTCCCAAGAGAAGGGTATGTTTCCTAATTTAATATGTAAAGACACATTATGTTTG
TTAGTCCATCTCACCCAACCTTGCCC

Exon 3:
CAATGCCTTCTTTTTGTCAAAGATGGGTGGAACA [A/G] GACCCTAAGGAAATTCTACATTCT
GTCTATGAGTGTATAGAGAAAACATGTGAGAACTTGGACAGCTCAATATTGATATTTCCAA
CATAAAAGGTATTTTAGTAGAATATTTTACCCACA

Exon 4:
TGTA AACGACGGCCAGTTGAGAGCTGTTTTCTGAAGTAGTTCCTACTTGTTAAATTTTTTG
ACTTCCTTCTGTTTAACTTTCTCTTTAAAGCTATTGGTGTGAGCAACCAGAGGGAAACCACT
GTAGTCTGGGACAAGATAACTGGAGAGCCTCTCTACAATGCTGTGGGTAAAGCTGTCATGCAT
GGATGTCAAATGTAGGGCCTTTCTTCACATTGCAA

Exon 5:
TGTA AACGACGGCCAGTTCCCTTGATAGTGATTTTCAGTAAGTTCTTATTTTTTTAAATGAAG
TTTTTCATGTATATTATTTTATTTTGGTCTATAGTGTGGCTTGATCTAAGAACCCAGTCTAC
CGTTGAGAGTCTTAGTAAAAGAATTCAGGAAATAATAACTTTGTCAAGGTAAGAATTTCTT
CAGAAGTATACTATAAGAATGTTTCTTTTTTTAAAAAAGTTTGCAGATTTCACTAGAAAGA
AGCATCTTATGGTACAATAGTTATTTGATACAATTTATAGAATCTTTTTCCCGGATAATTGA
GGCC

Exon 6:
TGTA AACGACGGCCAGTTTCTTTTGTGTTGGTGGTTTTGTTTTAAACTGTTACACTTTTTCAT
TTGCTAACTGAACTTCACAACCTGCTTTTAGTCCAAGACAGGCCTTCCACTTAGCACTTACTT
CAGTGCAGTGAACTTCGTTGGCTCCTTGACAATGTGAGAAAAGTTCAAAAGGCCGTTGAAG
AAAAACGAGCTCTTTTTGGGACTATTGATTCATGGCTTATTTGGGTATGTTTAAATATAATG
GATATATGGAGAAATTTTTTCAGAAATTTTTCTAGACTGCCTTGCCTATTGTTTCTACTAGC
AGGTCAGACTTTTTAATTAGCA

FIG. 7A

09694088.102000

Exon 7:

TGTA AAAACGACGGCCAGTTGTGCTCTGCTGATTATGACCCTTAACAATATGTAAATTAAATT
GCCAATAAGTACAAATTTAACCTGATTTTTTTTACTCTGCCTAGAGTTTGACAGGAGGAGTCA
ATGGAGGTGTCCACTGTACAGATGTAACAAATGCAAGTAGGACTATGCTTTTCAACATTCAT
TCTTTGGAATGGGATAAACAACTCTGCGAGTAAGTTCTGTTTTGCTCTAAATATAGTTTTCC
CAATACACTACCTATTTATAACCGAAATCTTAATATTTTCAGATGTCAGTGGAGCA

Exon 8:

TGTA AAAACGACGGCCAGTACAGTGTTAAATACCCAATCTTCTTGTTCAGATTTTTTGGTA
ATTCCAATGGAAATTTCTTCCAAATGTCCGGAGTTCTTCTGAGATCTATGGCCTAATGGTAAA
AAACAAACAAA [A/C] AAACAAAAAACACACCAAAAAACCAAAAAACAAACAAAAAAACC
TAATAATTAAAGTTTTTTTTATTACAAACAAGTTTACTATTTCATAATTCAAAGTCAACTGT
GTTATGTTTTGTGACTTAAAAACTTTACAGTCCTTTTTTACAATGG

Exons 9A and 9B

AAAGCTGGGGCCTTGGAAGGTGTGCCAATATCTGGGGTAAGTTTCATCACCAAGTGTCTCCC
CATCCCCACCCTTCCCCATGTTATGGCTTTTCTCCTCTTAGTTCATCAGTGTGCCTCTTTTT
AACTAGGGAAAAACAAGTAAAAGTTGCAAAATTGGANNNNTCTTGTTCTTACATGTCATACT
GTGGGCCATTGAGAACTTTTTGAATAAATTAATTTTAACTCTCCCTTCCCATACTATTATC
TTACATATTAACAAATGGTATTAACAAATGGGAAAAATGGCCAAATGGAGAAAATGCAAGGA
AATAGACAGTTCATTCTTTGATAAAATAAAAAATGAAAAATAAATCCTATGGCTCTTCTAAAA
AGAAAGTTAATACTATTGTATTAGTCAGTGTCTTTATTGTCAATTTATACTTTTCAGTGTTTA
GGGACCAGTCTGCTGCATTGGTGGGACAAATGTGCTTCCAGATTGGACAAGCCAAAAATAC
GTGAGTTTAAAGAAACAGACTTAAAAACCAATGCTGTTTTGTTTTTTCTACTTGGTGCTTTGA
ATAAGGAAAAGCTTTTGAAGTTCATCCAGGATGAAAATCAATAGCTTAATAGCTCCAATATG
CATATATACACTTTTTTACCATTTTTTTTATATCTTTAAATAAAATACAAAA
TGCCATATATATGCACACTGATGAAGCTTATAAAGACCTAAATTTGTAGGCTGGGCGCGG

Exons 10 and 11:

TTATTTGCTTTCAATAAAATTTGTCTTCTATTTCATTCTCCCTTCAACCATAGGTATGGAACAG
GATGTTTCTTACTATGT [A/G] ATACAGGCCATAAGGTTGGTTTTTTTAAATTAAAAAATTGA
TTTAAAAGTCTAAGTTCATCTAAATAATGCTTGAACATAATTTACTATTAAACAACTTTTAG
TCTTTAGCTTTTACTTAATCTTTATCAGGGTTTAAATTTAGAGCTCAATACAAAATTTGAATC
GTTCTAATAAGAACCATTTTAGACTCTTTGAATTTTATATGTGTGTTTTTAAATTGTGCTGGG
GGGAAATCTAGACTGAGACCTCATCAAATCTTAATGCAAATCTAATTTGAAACAAGGAATA
AACTTTTTTATACAGCTTAAATGTGTTCTTAATCTGATCGTTTTGACTGTAAGGATTTATTT
TAAAAATTGGTTTATTGATTGCATTATTTTGTACCTATGTTATTTTAACTTTAAAAAAAAGT
TCTCATGTTATCTTTTCATTTTCCACTACTGAAATCTTTTTTTTTTTCTTTCTTACAGTGTGT
ATTTTCTGATCATGGCCTTCTCACCACAGTGGCTTACAAACTTGGCAGAGACAAACCAGTAT
ATTATGCTTTGGAAGTAAGTTCTTTTTTAATCAATATGGATAATATGACAAACATTCAAAGCT
AATAAAAATCACAGAGTTTTCTAACACTTTTCTGGTAAATCTTAATACAGAGGACTCAAAAA
GTTCTGCTTTCTTGGCATTGATTGAGTTGAAGGAACCTGAAACTGATCTGGGTGTCAGGAC
TCACAGGAGACCTTGATTAGATTGGTTCCTCAGTTCTTATGCCAATTAATCATGTCACCTTA
GGCATATTACTTGAGAGCTCTACAATGTGAGGTTTTTTTTTTTTTTTATCTCTAAAGTTTAAAT
CGGATTAACGTGCTCTCTAACATTTCTTTCATCTTGAAAATCTTTGATTTTATAAATAAAA
TGCTCCAGTGTTCCAAAGAGAACCTGGGCACAAATAGGCAGAACAACTCTCTTCACTTGTC
TCCTCATAAAAATAAATTTTGTGTAACATTTTGATATAGAAAAGAAAGCGACGAGATTTATG
CCACTTATCACTGGAAACATTTGTTTTCAAACATTTTGTATGTTATAGTAGGAATATGCCAG
CCTAAGCCTATA

FIG. 7B

00020" 88046950

069708 - 10200

Exon 13:

Exons 14 and 15:

Exon 16:

Exon 17:

FIG. 7C

Exon 18:

TGTAACGACGGCCAGTTGGTTTGGTTTGCTTGACTGGAATCTCTTCTGCTTGGATGACCA
CAGGTGACCCTAGTATCTTCTGTAGTCTGCCCTTGGGCTTTTTTATAGTGAGTAGCATGGTA
ATGTTAATCGGAGCAAGGTACATCTCAGGTAGTTACTCTTTAAATTAGACAACTCTATTAG
TTAGCTTTAATGTTTTTCGTGTATAACTTAGCAGAAATTTTTCAGTGTTCATTCTTTCTG
TGTCTAGGAAGCTGGAAAATCAATTAAAGGTCTAATTAGTTAGACCAATTAATCTTTGGGGG
CAGTTAGAAGTAAGAAGTGTGACTCTGCTTACCCTTTTTAAATTTTAAATGTGATGACTTCT
TTAAGAGGGACTACATTCTGCTGTGAGCTGCAGCAATAAGCAAAAGTGAAAATACTAATATT
TAAATGACAGGACTTTTCACTGACTGCTGAAAGTTAAAGTATACTT

Exon 19:

AAAATTACTGGCTTAAATGGAAATGATGCTTCTTATTCTGTATGTTCCCATGAAAGTGAAAC
TTAAAAAAAATTTCATGATTAGGGTTTCATGAAAAGGCCCTTGTCTTATGAAAATTGAGAC
AGGTTGCATCTCTCTAAGCTAAAAGATGGGCTATGTGTCTAGAGTCTTAGACTTCTAAAATG
CATGTGGTCACTATATGTAGGTTATCTCTTCGGTGACATACACTGCAATTTGAGAGGGCTGC
AAATTGTTTGCCTTGGTAAACGATTAGCAACAGTGGCAATATTTGTTAATTTTGGAAATTGGC
CCTGTTTGTGCAATTTAATTGTGAGGCATGATTTAGAAATCATATGGACTTTCTAGCTTAA
TAAATGATTGAATCATCTGCATTGCTTTAACTCCTGAATTGTATGCATGTATTATTGACATA
TATGGTTTTTGTTCCTTCAGGTATTCCATAAAACCTACCAACTCATGGATTCCCAAGA
TGTGAGCTTTTACATAATGAAAGAACCAGCAATTCTGTCTCTTAATGCAATGACACTATT
CATAGACTTTGATTTTATTTATAAGCCACTTGCTGCATGACCCTCCAAGTAGACCTGTGGCT
TAAAATAAAGAAAATGCAGCAAAAAGAATGCTATAGAAATATTTGGTGGTTTTTTTTTTTTT
TAAACATCCACAGTTAAGGTTGGGCCAGCTACCTTTGGGGCTGACCCCTCCATTGCCATAA
CATCCTGCTCCATTCCCTCTAAGATGTAGGAAGAATTCGGATCCTTACCATTGGAATCTTCC
ATCGAACATACTCAAACACTTTTGGACCAGGATTTGAGTCTCTGCATGACATATACTTGATT
AAAAGGTTATTACTAACCTGTTAAAAATCAGCAGCTCTTTGCTTTTAAAGAGACACCCTAAAA
GTCTTCTTTTCTACATAGTTGAAGACAGCAACATCTTCACTGAATGTTTGAATAGAAACCTC
TACTAAATTATTAATAATAGACATTTAGTGTTCTCACAGCTTGGATATTTTCTGAAAAGTTA
TTTGCCAAAAGTGAATCCTTCAGATGTTTTCCATGGTCCCACTAATTATAATGACTTTCTG
TCTGGGTCTTATAGGAAAAGATACTTTCTTTTTTCTTCCATCTTTCCTTTTTTATATTTTAA
CTTTGTATGTATAACATACATGCCTATATATTTTATACACTGAGGGAGCCCATTTATAAATA
AAGACCACATTATATTCAGAAGGTTCTAACAGGG

FIG. 7D

TABLE 1

Characteristics of carriers of the N288D GK gene mutation
and of their unaffected relatives

	Men			Women		
	N288D carriers	Unaffected relatives	p	N288D carriers	Unaffected relatives	p
N	18	18		14	14	
Age (years)	46,4±14,2	42,0±18,8	0.32	44,9±13,5	43,7±17,8	0.87
Uncorrected triglyceride (mmol/L) ⁽¹⁾	6,26±1,13	2,05±0,54	<0.0001	2,84±1,20	1,30±0,65	0.0002
Glycerol (mmol/L)	3,99±0,71	0,10±0,04	<0.0001	0,54±0,14	0,10±0,02	<0.0001
Corrected triglyceride (mmol/L) ⁽¹⁾	2,27±0,75	1,95±0,53	<0.0001	2,31±1,22	1,19±0,67	0.03
Free fatty acid (mmol/L)	0,77±0,22	0,57±0,25	0.01	1,29±0,35	0,76±0,17	0.0004
Fasting glucose (mmol/L)	5,2±0,74	4,8±0,31	0.13	5,0±0,7	4,6±0,3	0.10
2h glucose following OGTT (mmol/L)	7,9±3,1	5,8±1,6	0.02	7,0±5,1	5,0±2,1	0.04
Fasting insulin (mU/L) ⁽¹⁾	13,3±14,0	15,1±14,8	0.62	12,2±13,1	9,0±3,4	0.60
Waist girth (cm)	97,7±9,3	88,1±12,3	0.01	88,5±3,3	79,3±5,3	0.03
Body mass index (kg/m ²)	27,9±4,1	24,9±3,9	0.03	28,1±5,5	23,1±2,3	0.001
% total body fat	27,1±7,2	22,9±7,6	0.01	46,3±3,1	33,9±11,3	0.001

(1) geometric mean, p value after log transformation

Figure 8

Fasting plasma glycerol concentration (mmol/L) in the initial cohort of 1056 individuals, by risk factor of glucose intolerance and diabetes mellitus

		No.	Glycerol geometric mean \pm SD	p
Gender				
	men	717	0.065 \pm 0.081	
	women - premenopausal	137	0.071 \pm 0.093	<0.0001
	- menopausal	202	0.099 \pm 0.085	
Age (Y)				
	<50	486	0.071 \pm 0.082	
	50 - 60	408	0.076 \pm 0.106	0.0015
	>60	165	0.083 \pm 0.053	
Fasting glucose (mmol/L)				
	< 5.2	449	0.063 \pm 0.080	
	5.2 - 5.9	356	0.070 \pm 0.090	<0.0001
	6.0 - 6.9	271	0.090 \pm 0.100	
Fasting insulin (UI)				
	<15	637	0.067 \pm 0.082	0.02
	\geq 15	419	0.086 \pm 0.101	
2 hours glucose (mmol/L)				
	<7.3	572	0.062 \pm 0.071	
	7.3 - 11.0	283	0.081 \pm 0.101	<0.0001
	\geq 11.1	201	0.102 \pm 0.110	
Triglyceride (mmol/L)				
	\leq 2.2	389	0.057 \pm 0.062	<0.0001
	>2.2	667	0.082 \pm 0.103	
Free fatty acid (mmol/L)				
	<0.6	589	0.066 \pm 0.054	<0.0001
	\geq 0.6	467	0.111 \pm 0.112	
Body mass index (kg/m ²)				
	\leq 27	423	0.060 \pm 0.087	<0.0001
	>27	623	0.079 \pm 0.097	

Figure 9

TABLE 3. Multivariate analysis of the relationships of fasting plasma glycerol concentration with impaired glucose tolerance (2h glucose 7.3-11.0 mmol/L following a 75 g oral load) before and after adjustment for covariates identified in

	Model 1	Model 2	Model 3	Model 4
Glycerol (log)				
β	1.75	1.62	1.46	0.77
Odds ratio	5.76	5.42	4.33	2.41
p	<0.0001	<0.0001	<0.0001	0.01
Triglyceride (log)				
β		0.54	0.35	0.11
Odds ratio		1.75	1.42	1.11
p		0.02	0.11	0.63
Body mass index (kg/m²)				
β			0.10	0.03
Odds ratio			1.10	1.03
p			<0.0001	0.01
Fasting insulin (log)				
β				0.37
Odds ratio				1.31
p				0.39
Fasting glucose (mmol/L)				
β				1.13
Odds ratio				2.65
p				<0.0001
Free fatty acid (log)				
β				1.12
Odds ratio				4.13
p				0.007

Odds ratios are expressed as the increase in the risk of 2h glucose ≥7.3 mmol/L following a 75 g oral charge, associated with a 1-SD increase in the variables studied. β denotes the standardized estimate which is the parameter estimate of each variable in the multivariate logistic model. All models included age and gender as covariates. Otherwise, only the variables included in each model are shown. Subjects with severe hypertriglyceridemia due to the N788D mutation in the